IV. Sample Collection and Handling

A. Rationale for sample collection. When an animal develops rabies (most often when the bite of another animal transfers rabies-virus-laden saliva to the wound), rabies virus moves transneuronally from the site of entry to the spinal cord and brain. Patterns of virus spread within the central nervous system suggest that a thorough examination of the brain stem is critical to rabies diagnosis. Viral antigen is widespread in the brain of most animals positive for rabies, but because spread may also be unilateral, especially in larger animals (Figure 1), a negative finding for rabies can be made only if a complete cross-section of the brain stem is examined. Examination may be made at the level of the pons, medulla, or midbrain.

Figure 1. DFA test of a transverse section (cross-section) of brain stem from a rabies infected donkey showing unilateral virus spread (200X magnification, frozen section).

Cerebellar tissue should also be included in a rabies test. Although brain stem is the tissue most reliably found to contain viral antigen, the characteristic size and shape of intracytoplasmic inclusions produced as rabies virus accumulates in the large neurons of follar regions of the cerebellum are easily detected and recognized by DFA. Inclusion of this tissue yields a more confident diagnosis than examination of brain stem alone. Although the hippocampus was once the tissue of choice for histologic tests for Negri bodies, hippocampus is of limited additional value when brain stem and cerebellum are examined. If the cerebellum is missing from tissue submitted for rabies testing, however, a negative finding may be made from examination of brain stem and hippocampus. While a negative finding for rabies can be made only if brain stem tissue is among the tissues examined, incomplete specimens should be tested, if possible. Specific staining in any tissue reacted with anti-rabies antibody is diagnostic of rabies infection.

Virus is present in the saliva of an infected animal only after virus proliferation in the central nervous system and subsequent centrifugal spread from the brain to the salivary glands. A negative DFA test for the presence of rabies virus in brain tissue assures that contact with saliva of a biting animal could not have transmitted rabies. Because virus may not spread to all salivary glands and may be present only intermittently in saliva, negative tests of salivary glands or saliva cannot rule out rabies infection.

B. Shipment of samples. Because rabies prophylaxis is usually delayed pending a laboratory report, specimen transit time to the laboratory should be as short as possible, preferably within 48 hours. A fresh, unfixed brain sample is critical to a rapid and accurate diagnosis of rabies. Refrigeration will preserve a sample for at least 48 hours. Freezing of the sample for transit will not reduce the sensitivity of the test, but may introduce additional testing delays and impede recognition and dissection of appropriate test samples. Repeated freeze-thaw cycles may reduce test sensitivity and should be avoided. Biocontainment during specimen transport is critical, to prevent both contamination of the outside of the package and cross-contamination between samples within the package.

C. Unacceptable deterioration or decomposition of a sample is a qualitative assessment of the condition of each sample upon arrival in the lab. Substantial green color, liquefaction, desiccation, or an unrecognizable gross anatomy can indicate an unsatisfactory sample. A substantial loss of tissue during staining and washing or the presence of bacteria on the stained slide may also indicate sample deterioration. If negative results are obtained on deteriorated tissue, the test report should state only that the condition of the sample is such that tests cannot rule out the presence of rabies virus in the specimen. The negative findings should not be mentioned, since this is often misinterpreted as a negative diagnosis. Positive test results are reported as such.

D. Chemical fixation (e.g., formalin) can alter tissue to make a sample unsuitable for testing. Methods for testing fixed tissue exist, but these tests are performed by other than standard DFA methods on tissues prepared by controlled fixation procedures. These methods are not available in all laboratories and referral to reference laboratories will delay test results.

E. Laboratory handling of samples. Samples submitted to the laboratory may be a complete carcass, an intact head, or dissected brain tissue.

Dissected brain tissue must include a complete cross section of the brain stem and either cerebellum or hippocampus. All material submitted with a sample, including the carcass, should be held frozen until the test is completed and results reported. A single additional freeze-thaw cycle will have no effect on rabies-specific staining if repeat testing of the sample should be required, and the freeze-thaw cycle may eliminate or reduce some non-specific staining. Retention of the carcass is necessary to verify the identity of an animal in the case of unusual test results, and to identify a wild animal to species. Sample identifiers (accession numbers) should be used to label boxes and all items accompanying the sample. All necropsy and tissue processing must include proper identification of each sample and avoidance of any practice that could lead to cross contamination of samples. Each specimen should be handled on a clean work surface with new disposable gloves. All instruments used during necropsy, dissection, and slide preparation must be thoroughly disinfected by boiling or autoclaving followed by thorough washing before reuse. Instruments not in use should be kept in closed storage. Only those instruments in use for processing a single sample should be exposed. Frozen reference material taken at necropsy should be retained in the laboratory for all test samples. Most laboratories maintain test samples for 2 to 6 months, but representative positive samples should be maintained for longer periods for use as controls, for epidemiologic typing, and for other purposes. Storage containers for reference material must be large enough that reserved portions of brain stem and cerebellum (and hippocampus, if desired) remain as recognizably separate pieces and allow complete cross sections to be made if repeat testing is required. A video describing necropsy procedures is available from the CDC.

V. Preparation of Impression Slides / Smears

A. Sampling. While a positive finding of rabies virus antigen in any tissue is diagnostic of rabies infection, a negative finding for rabies can be made only if the diagnostic test includes examination of at least two areas of the brain: brain stem and preferably cerebellum (Figures 2,3,4,5,6,7,8,9,10,11,12,13). A complete cross section of the brain stem is required. If brain stem is unavailable and other brain tissues are negative,the sample must be considered **unsatisfactory** for testing. If cerebellum is unavailable, a diagnosis may be made by examination of brain stem and hippocampus (Figures 14 and 15). Each brain area is tested

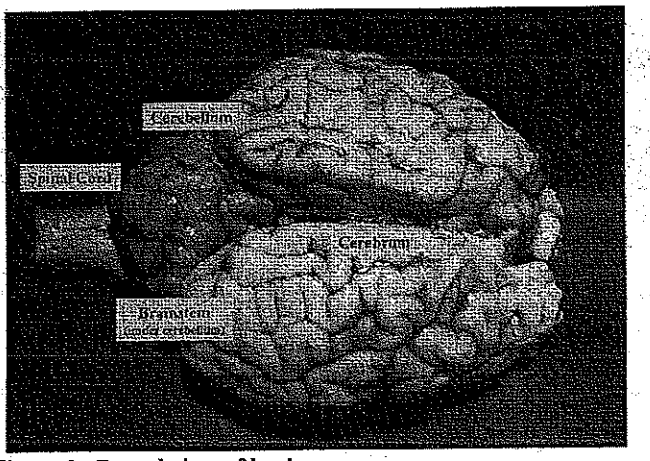

Figure 2. Dorsal view of brain

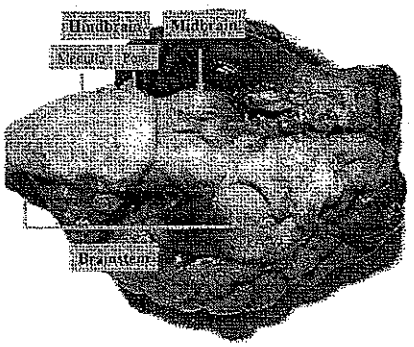

Figure 3. Ventral view of brain showing areas of brain stem suitable for rabies testing

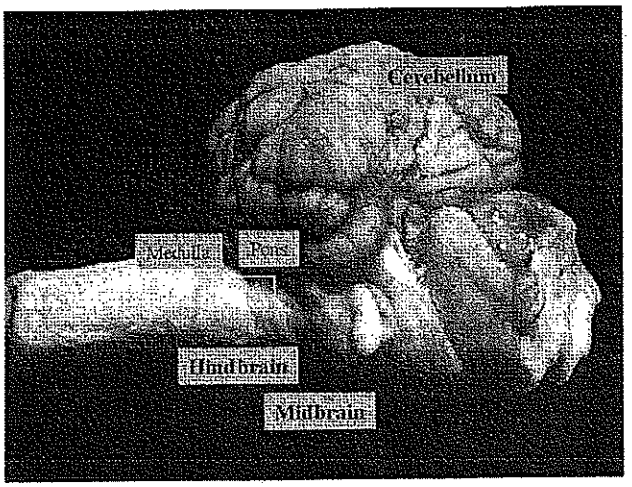

Figure 4. Lateral view of brain with cerebrum removed to show the extension of brain stem beneath the cerebellum. A rabies diagnosis should include an observation of the cut surface of a cross section of the brain stem (through the medulla, pons, or midbrain area) and the cerebellum (through each hemisphere and the vermis). For example, a cross section of the midbrain area (dashed line) would include all tissues necessary for rabies diagnosis